REMARKS

Claims 1-9 are presently pending in this application. Claim 1 has been amended to further define the invention. The amendment is intended to make clear that the amplificates, not the primers, have a length of less than 75 nucleotides. This clarification was necessitated by the misunderstanding reflected in the Examiner's statement in the last paragraph on page 5 of the Office Action. The amendment is supported by the specification and does not introduce new matter. A "Version with markings to show changes made" is attached herewith.

The specification has been amended to introduce section headings in accordance with the Examiner's suggestion. These amendments do not introduce new matter.

Reconsideration of the application as amended is respectfully requested.

I. The Rejection Under 35 U.S.C. § 112, Second Paragraph, Should Be Withdrawn

Claims 1-9 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject invention. More specifically, the Examiner considers certain notations in step (a) of Claim 1 unclear.

Claim 1 is directed to a method for the detection of a nucleic acid, which is supported by the specification at page 23, line 16 to page 24, line 12 and the drawings. In particular, the Applicants invite the Examiner's attention to Figures 1 and 3 for a schematic illustration of the claimed invention. The notations of the nucleic acid sequences in Claim 1 are identical to those used in these drawings. For example, Fig. 1 shows a double-stranded nucleic acid as a target, which is divided into three contiguous regions of nucleotide sequences. Reading from the 5' to 3' direction, the 5'-most sequence of the first strand is denoted A. The sequence of the region 3' to A is denoted B. The 3'-most sequence is denoted C. On the complementary strand, the sequences that are the complements of regions A, B and C are denoted A', B' and C', respectively.

Step (a) of Claim 1 recites the production of a plurality of amplificates with the aid of two primers from a target nucleic acid. In this process, one primer binds to region A of the first strand of the target nucleic acid, while the second primer binds to region C' of the complementary strand. As shown in Fig. 1, C' is the complement of C which is located on the 3' end of the first strand (i.e. C is 3' from A). This description of the use of two primers for amplifying a target nucleic acid is fully consistent with standard amplification methods such as the polymerase chain reaction (PCR). In PCR, each primer binds to one of the two complementary strands, and the two primers bind at opposite ends.

Additionally, step (a) of Claim 1 recites that a probe with a nucleotide sequence denoted D binds to the region B of the target nucleic acid or B' on the complementary strand. This interaction between the probe (D) and the specific region of the amplificates (B or B') is illustrated in Fig. 3I. In that connection, the Applicants submit that the probe (D) in Claim 1 is meant to bind to B or B', but not both. Since both strands of a double-stranded nucleic acid are amplified, the amplificates may be detected by a probe which binds to either strand; i.e. B or B'. The notation of D for the probe sequence is not meant to indicate that D is complementary to both B and B' at the same time.

It is noted that Claim 1 does not employ any notations for the sequences of the primers. Instead, primers are described as binding to certain regions in a double-stranded nucleic acid referred to as A and C'. However, the nucleotide sequence of the probe in Claim 1 is given the notation D, and this sequence detects the amplificates by binding to either region B or B'.

Turning to dependent Claims 3 and 6-8, the Examiner questions how a primer or probe can hybridize to a nucleic acid for which it is not specific. The term "specificity" is commonly used in the art to refer to the ability of a nucleic acid to hybridize uniquely to a target sequence in a way that distinguishes the target from other unrelated sequences. A non-specific primer or probe may hybridize to sequences other than the target sequence, but it does not imply that there is a reduction in its ability to hybridize to the target sequence. In other words, a non-specific primer or probe is capable of hybridizing to a target sequence, but it also may hybridize to other sequences.

In the context of the present invention, a non-specific primer or probe can still result in the specific detection of a target sequence when the primers and probe are used in combination in an assay. The specification at page 36, line 10 to page 37, line 24 teaches in detail how the overall specificity of the claimed method can be retained even though certain individual primers or probe is not specific for a target sequence.

In view of the foregoing, Applicants submit that the amended independent Claim 1, and Claims 3 and 6-8 are both clear and definite. Withdrawal of the rejection of Claims 1-9 under Section 112, second paragraph, is respectfully requested.

II. The Rejection Under 35 U.S.C. § 103(a) Should Be Withdrawn

Claims 1-9 are rejected under 35 U.S.C. § 103(a) as unpatentable over Livak (U.S. Patent No. 5,538,848).

The presently claimed invention is directed to a method for detecting a nucleic acid by amplifying a target nucleic acid and detecting the amplificates having a length of less than 75 nucleotides by a probe with a reporter group and a quencher group. Livak describes a method for detecting nucleic acid amplification using self-quenching flourescence probe. With respect to nucleic acid amplification, Livak does not teach or suggest amplificates having a length of less than 75 nucleotides which are detected in its method. In that connection, Livak only states that its detection method may be used in conjunction with the amplification of a target polynucleotide by PCR (column 4, lines 15-21), and it refers to general references for such teachings.

One of the well known general references on PCR is PCR Primer, A Laboratory Manual, Cold Spring Harbor Laboratory, NY (1995). In this reference, Dieffenbach et al. describes certain principles for PCR primer design at pages 133-142, a copy of this reference is enclosed in the accompanying Information Disclosure Statement as reference AF. With regard to the length of a PCR amplified product, Dieffenbach teaches that it is generally in the range of 150-1,000 base pairs (page 138, first full paragraph). Furthermore, it states that the length of an amplification product in a clinical assay may be smaller, in the range of 120-300 base pairs (page 138, second full paragraph). Thus, this PCR reference does not teach any amplified product smaller than 120 nucleotides in length.

Additionally, a working example in Livak at column 7, lines 33-36 discloses the use of primers to amplify a 295 base pair segment of a human actin gene. Therefore, Livak does not teach or suggest the detection of an amplificate of less than 75 nucleotides. Since it is well known in the art that short amplified products present more restrictions for the design of primers and probes, a person skilled in the art would not have had the motivation to generate amplificates of less than 75 nucleotides for detection by the method of Livak.

Against this background, the present invention demonstrates that short (less than 75 nucleotides) amplified products produce certain unexpected advantages when they are detected by a probe containing a reporter group and a quencher group. Such advantages include reduced background signals, increased reproducibility and increased specificity (page 39, lines 17-33).

Since Livak neither suggests nor provides a motivation for one skilled in the art to practice the claimed invention, it does not render the claimed invention obvious. The rejection under Section 103 should be withdrawn.

III. Conclusion

In view of the foregoing amendments and remarks, the Examiner's rejections have been obviated or overcome. Applicants submit that all presently pending claims are in condition for allowance, and an early allowance is earnestly sought.

Respectfully submitted:

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. (Twice Amended) A method for the detection of a nucleic acid comprising the steps:

(a) - producing a plurality of amplificates of a section of the nucleic acid with the aid of two primers, one of which can bind to a first binding sequence (A) of one strand of the nucleic acid and the other can bind to a second binding sequence (C') which is essentially complementary to a sequence C which is located in the 3' direction from A and does not overlap A, in the presence of a probe with a binding sequence D which can bind to the third sequence (B) located between the sequences A and C or to the complement (B') thereof, wherein this probe contains a reporter group and a quencher group, using a polymerase having 5' nuclease activity, and

(b) - detecting the nucleic acid by measuring a signal which is caused by the release of the reporter group, wherein the amplificates [formed with the aid of the primers] have a length of less than 75 nucleotides.

Specific and sensitive nucleic acid detection method

FIELD OF THE INVENTION

The invention concerns a method for detecting nucleic acids in which a section of these nucleic acids is amplified whereby this section must fulfil certain conditions with regard to its base sequence and it also concerns a reagent kit containing two primers and a probe which define this section.

BACKGROUND OF THE INVENTION

One of the most frequently employed molecular-biological methods for detecting nucleic acids is hybridization with sequence-specific probes to detect homologous nucleic acid sequences. The detection of nucleic acid sequences is important for basic research but is of particular importance in various fields of application e.g. in the fields of medical diagnostics, forensic diagnostics, food diagnostics, environmental diagnostics, plant protection and veterinary medicine.

Either oligonucleotides (short DNA or RNA) or polynucleotides (longer DNA or RNA) are used as probes for this. An advantage of the shorter probes compared to the longer probes is that they have a better sequence selectivity due to the shorter hybridization region but they have the disadvantage of lower sensitivity. An improved sensitivity and sequence selectivity is achieved with PNA probes (peptide nucleic acids, e.g. WO 92/20702) since these probes have a higher binding affinity for nucleic acids (higher Tm) and are characterized by a higher base discrimination (ΔTm). Probes can additionally carry marker groups for nucleic acid detection which are suitable either for capturing and/or detecting hybrid complexes of the probe and nucleic acid to be detected.

plateau effects of the participating enzymes and substrates which are reached earlier with longer amplicons. A further disadvantage of longer nucleic acid amplification products is an increased competition between the amplicon complementary strand and the detector or capture probe and thus a reduced sensitivity. A further disadvantage is the increasing chance of unspecific binding due to the additional sequences resulting in an increased background and thus lower sensitivity (lower signal-noise ratio). A further disadvantage when the nucleic acid amplification product is bound to carrier-bound capture probes is steric and kinetic hindrance of longer nucleic acid molecules; consequently nucleic acid amplification products of the former lengths have been preferably fragmented before binding to the capture probe. An additional disadvantage is the increased susceptibility to fragmentation within the amplicon sequence and thus destruction of the nucleic acid amplification unit; this leads to a lower reproducibility. An additional disadvantage is that longer nucleic acid amplification products hybridize less specifically at low test temperatures of e.g. 37°C which are preset in conventional nucleic acid analyzers since there is a larger difference to the melting temperature. A further disadvantage of five part nucleic acid amplification products when detecting several different nucleic acid amplification products is that different nucleic acid amplification lengths are formed which make it more difficult to carry out a multiplex test.

SUMMARY OF THE INVENTION

The aim of the present invention was to provide an alternative detection method for nucleic acids which has advantages over the previously described methods.

The invention also concerns a reagent kit for carrying out this method.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows schematically the notation used in the present description for the regions on the nucleic acid to be detected.

Fig. 2 shows the corresponding notation for the elongation products of the primers formed as intermediates as well as for the amplificates (amplicons). It also shows that the amplificates can have one or several additional regions Y which are outside the region that contains the sequence information derived from the nucleic acid to be detected.

Fig. 3 shows schematically the arrangement of the binding sequences of the primers and probe in the present invention. There are various alternatives I to VI depending on whether and how the binding sequences overlap. Only one strand of the amplificate is shown in each case. The same arrangement (only complementary) can be constructed for a second strand of the amplificate. The picture is similar for the elongation products formed as intermediates. Cases V and VI show that, in addition to the binding sequence D, the probe contains additional regions X which can be the same or different and do not form base pairs with the amplificate. The prior art case is shown as VII for comparison; the sequences Z represent the additional sequences of the five part amplicon.

Fig. 4 shows a particularly suitable region of the HCV genome for performing the method according to the invention and a sequence from which the primer and probe sequences are preferably selected. This second sequence

is taken from the non-human pathogenic virus HGBV-B. The selected primer and probe sequences are therefore sequences that are not specific for HCV (M. Med. Virol. 48, 60-67).

Fig. 5 shows further preferred primers and probes.

Fig. 6 and 7 show additional primers and probes.

Fig. 8 shows the particularly preferred HCV region which should completely or partially be provided as a template for the amplification. It should not contain adjoining sequences.

DETAILED DESCRIPTION OF THE INVENTION

Nucleic acids which can be detected with the method according to the invention can be of any origin such as nucleic acids of viroidal, viral, bacterial or cellular origin or from yeasts or fungi. Samples which contain the nucleic acid sequences to be detected or complements thereof are for example human, animal, bacterial or plant liquids or liquids from yeasts or fungi, excrements, smears, cell suspensions, cultures or tissue, cell or liquid biopsies. The nucleic acids are preferably present in solution. In order to realize the full advantages of the method according to the invention it has proven to be advantageous when the nucleic acid to be detected has a size of at least 40 bp. However, the nucleic acid can also be a nucleic acid prepared by cloning, amplification, or in vitro or in vivo replication.

The nucleic acid to be detected can be single-stranded (especially in the case of RNA) or completely or partially double-stranded (especially in the case of